0303459A3 Church Mulitplex sequencing Feb. 15, 1989.
Canadian Patent Application 2,036,946 Deugau et al. Indexing linkers. Oct. 7, 1991.
Crick et al, "Codes without commas," Proc. Natl. Acad. Sci., 43: 416–421 (1957).
Ohlmeyer et al, "Complex synthetic chemical libraries indexed with molecular tags," Proc. natl. Acad. Sci., 90: 10922–10926 (1993).
Maskos and Southern, "Oligonucleotide hybridizations on glass supports: a novel linker for oligonucleotide synthesis and hybridization properties of oligonucleotides synthesized in situ," Nucleic Acids Research, 20: 1679–1684 (1992).
Matthews and Kricka, "Analytical strategies for the use of DNA probes," Anal. Biochem. 169: 1–25 (1988).
Broude et al., "Enhanced DNA sequencing by hybridization," Proc. Natl. Acad. Sci. 91: 3072–3076 (1994).
Nielsen et al., "Synthesis methods for the implementation of encoded combinatorial chemistry," J. Am. Chem. Soc. 115: 9812–9813 (1993).
Needels et al, "Generation and screening of an oligonucleotide–encoded synthetic peptide library," Proc. Natl. Acad. Sci., 90: 10700–10704 (1993).
Chetverin et al, "Oligonucleotide arrays: New concepts and possibilities," Biotechnology, 12: 1093–1099 (1994).
Yang and Youvan, "A prospectus for multipspectral–multiplex DNA sequencing," Biotechnology, 7: 576–580 (1989).
Church et al, "Multiplex DNA Sequencing," Science, 240: 185–188 (1988).
Beck et al, "A strategy for the amplification, purification, and selection of M13 templates for large–scale DNA sequencing," Analytical Biochemsitry, 212: 498–505 (1993).

(List continued on next page.)

Primary Examiner—George C. Elliott
Assistant Examiner—Scott D. Priebe
Attorney, Agent, or Firm—Stephen C. Macevicz

[57] **ABSTRACT**

The invention provides a method of tracking, identifying, and/or sorting classes or subpopulations of molecules by the use of oligonucleotide tags. Oligonucleotide tags of the invention each consist of a plurality of subunits 3 to 6 nucleotides in length selected from a minimally cross-hybridizing set. A subunit of a minimally cross-hybridizing set forms a duplex or triplex having two or more mismatches with the complement of any other subunit of the same set. The number of oligonucleotide tags available in a particular embodiment depends on the number of subunits per tag and on the length of the subunit. An important aspect of the invention is the use of the oligonucleotide tags for sorting polynucleotides by specifically hybridizing tags attached to the polynucleotides to their complements on solid phase supports. This embodiment provides a readily automated system for manipulating and sorting polynucleotides, particularly useful in large-scale parallel operations, such as large-scale DNA sequencing, mRNA fingerprinting, and the like, wherein many target polynucleotides or many segments of a single target polynucleotide are sequenced simultaneously.

4 Claims, 6 Drawing Sheets